Carbonaceous and nitrogenous disinfection by-product formation from algal organic matter

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Abstract

Seasonal algal blooms in drinking water sources release intracellular and extracellular algal organic matter (AOM) in significant concentrations into the water. This organic matter provides precursors for disinfection by-products (DBPs) formed when the water is subsequently chlorinated at the final disinfection stage of the potable water treatment process. This paper presents results of AOM characterisation from five algal species (three cyanobacteria, one diatom and one green) alongside the measurement of the DBP formation potential from the AOM of six algal species (an additional diatom). The character was explored in terms of hydrophilicity, charge and protein and carbohydrate content. 18 DBPs were measured following chlorination of the AOM samples: the four trihalomethanes (THMs), nine haloacetic acids (HAAs), four haloacetonitriles (HANs) and one halonitromethane (HNM).

The AOM was found to be mainly hydrophilic (52 and 81\%) in nature. Yields of up to 92.4 µg mg\textsuperscript{-1} C carbonaceous DBPs were measured, with few consistent trends between DBP formation propensity and either the specific ultraviolet absorbance (SUVA) or the chemical characteristics. The AOM from diatomaceous algae formed significant amounts of nitrogenous DBPs (up to 1.7 µg mg\textsuperscript{-1} C). The weak trends in DBPFP may be attributable to the hydrophilic
nature of AOM, which also makes it more challenging to remove by conventional water
treatment processes.

**Keywords**
Algae, trihalomethanes, haloacetic acids, haloacetonitriles, characterisation

**Abbreviations**

<table>
<thead>
<tr>
<th></th>
<th>Abbreviation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>AOM</td>
<td>algal organic matter</td>
<td></td>
</tr>
<tr>
<td>BCAA</td>
<td>bromochloroacetic acid</td>
<td></td>
</tr>
<tr>
<td>BDCAA</td>
<td>bromodichloroacetic acid</td>
<td></td>
</tr>
<tr>
<td>C-DBPs</td>
<td>Carbonaceous DBPs</td>
<td></td>
</tr>
<tr>
<td>DBAA</td>
<td>dibromoacetic acid</td>
<td></td>
</tr>
<tr>
<td>DBCAA</td>
<td>dibromochloroacetic acid</td>
<td></td>
</tr>
<tr>
<td>DBPs</td>
<td>disinfection by-products</td>
<td></td>
</tr>
<tr>
<td>DCAA</td>
<td>dichloroacetic acid</td>
<td></td>
</tr>
<tr>
<td>DCAN</td>
<td>dichloroacetonitrile</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
<td></td>
</tr>
<tr>
<td>DWI</td>
<td>Drinking Water Inspectorate</td>
<td></td>
</tr>
<tr>
<td>DXAA</td>
<td>dihalogenated acetic acids</td>
<td></td>
</tr>
<tr>
<td>ECD</td>
<td>electron capture detection</td>
<td></td>
</tr>
<tr>
<td>EOM</td>
<td>extracellular organic matter</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>MBAA</td>
<td>monobromoacetic acid</td>
<td></td>
</tr>
<tr>
<td>MCAA</td>
<td>monochloroacetic acid</td>
<td></td>
</tr>
<tr>
<td>MXAA</td>
<td>monohalogenated acetic acids</td>
<td></td>
</tr>
<tr>
<td>N-DBPs</td>
<td>Nitrogenous DBPs</td>
<td></td>
</tr>
<tr>
<td>NOM</td>
<td>natural organic matter</td>
<td></td>
</tr>
<tr>
<td>OM</td>
<td>organic matter</td>
<td></td>
</tr>
<tr>
<td>SUVA</td>
<td>specific ultraviolet absorbance</td>
<td></td>
</tr>
<tr>
<td>TCAA</td>
<td>trichloroacetic acid</td>
<td></td>
</tr>
<tr>
<td>TCAN</td>
<td>trichloroacetonitrile</td>
<td></td>
</tr>
<tr>
<td>TCM</td>
<td>trichloromethane</td>
<td></td>
</tr>
<tr>
<td>TCNM</td>
<td>trichloronitromethane</td>
<td></td>
</tr>
<tr>
<td>THMs</td>
<td>trihalomethanes</td>
<td></td>
</tr>
<tr>
<td>TPI</td>
<td>transphilic organic fraction</td>
<td></td>
</tr>
<tr>
<td>TXAA</td>
<td>trihalogenated acetic acids</td>
<td></td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
<td></td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet absorbance at 254 nm</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
<td></td>
</tr>
</tbody>
</table>
Chlorination of drinking water is known to cause the formation of disinfection by products (DBPs) which are a health concern (Richardson, 2003). Carbonaceous DBPs (C-DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs) are formed when the organic matter (OM) in the water reacts with chlorine. THMs are widely regulated at 80, 100, 100 and 250 µg L$^{-1}$ for the sum of four THMs in the USA, Europe, Canada and Australia respectively (USEPA, 1998, Health Canada, 2012, EU, 1998, NHMRC, 2011). Nitrogenous DBPs (N-DBPs) such as haloacetonitriles (HAN) and halonitromethanes (HNM) are also of health concern and have been shown to be more cytotoxic and genotoxic than C-DBPs (Plewa, 2002). They are not regulated but some (dichloroacetonitrile and dibromoacetonitrile at 20 and 70 µg L$^{-1}$ respectively) are incorporated in the WHO drinking water guidelines (WHO, 2006). Although in the EU THMs are the only chlorinated DBPs regulated, the approach to meeting the regulation is becoming risk based; regulations make clear the duty to minimise DBPs as a whole.

The most studied type of OM is terrestrial or natural organic matter (NOM) which varies seasonally by, for example, leaching from soil (Thibodeaux and Aguilar, 2005). Advances in water treatment and an understanding of NOM behaviour have enabled sufficient and enhanced removal of organic DBP precursors to minimise DBP formation. The seasonality of the NOM quantities and character can be addressed with enhanced coagulation controlled through UV$_{254}$ (Fabris et al., 2013) and zeta potential (Sharp et al., 2006) monitoring. The yield of DBPs (µg/mg C or µg/UV$_{254}$) from NOM has been shown to correlate with dissolved organic carbon (DOC) and UV absorbance at 254 nm (UV$_{254}$); reported yield values for THMs and HAAs have ranged from 61 to 124 µg/mg C across various studies (Table 2).
A less extensively studied source of OM is from algae, generating dissolved organic carbon (DOC) levels of 1-25 mg L$^{-1}$ (Nguyen et al., 2005) from algal organic matter (AOM) (Pivokonsky et al, 2016). Besides contributing to the organic carbon content in water, algal cells contain organic nitrogen in the form of polysaccharides, proteins, peptides, amino sugars and other trace organic acids (Huang et al., 2009). AOM arises (a) extracellularly via metabolic excretion, forming extracellular organic matter (EOM) or (b) intracellularly due to autolysis of cells, forming intracellular organic matter (IOM). AOM is known to comprise proteins, neutral and charged polysaccharides, nucleic acids, lipids and small molecules, of which polysaccharides can comprise up to 80–90% of the total release. The IOM proportion increases with increasing age of the algae system (Henderson et al., 2008). EOM and IOM are of interest when studying the DBPs formed when algae arises in source waters, since they may be recalcitrant to water treatment (Henderson et al., 2010).

The study of THM and HAA formation from AOM (Wachter and Andelman, 1984; Schmidt et al., 1998; Nguyen et al., 2005; Huang et al., 2009; Zhou et al., 2014) has generally been focused on the chlorination of water containing algal cells (Hong et al, 2008; Huang et al. 2009; Laio et al, 2015). Both algal cells and AOM can potentially generate significant amounts of THMs and HAAs. There has also been some work on the formation of nitrogenous DBPs, such as HANs, from chlorination of algal cells and/or AOM and its fractions (Oliver, 1983; Fang et al., 2010; Zhou et al., 2014). As with NOM, AOM can be fractionated according to both size and chemistry, with studies indicating the hydrophilic (HPI) chemical fraction to dominate over the transphilic (TPI) and hydrophobic (HPO) fractions regardless of the status of growth in the cell life cycle (Table 1). Studies of fraction yield, the mass of chlorinated DBP formed per unit mass of organic carbon in μg DBP per mg C, indicate similar DBP formation trends in AOM
as reported for NOM, the most reactive fractions being those at higher molecular weight (Lui et al, 2012) and hydrophobicity (Zhou et al, 2014).

Table 1: % distribution of AOM between the three chemical fractions, *Microcystis aeruginosa*

<table>
<thead>
<tr>
<th>Growth phase</th>
<th>HPO</th>
<th>TPI</th>
<th>HPI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponential</td>
<td>27</td>
<td>4</td>
<td>69</td>
<td>Pivokonsky et al, 2014</td>
</tr>
<tr>
<td>Exponential</td>
<td>24</td>
<td>9</td>
<td>67</td>
<td>Zhou et al, 2014</td>
</tr>
<tr>
<td>Exponential</td>
<td>2</td>
<td>23</td>
<td>75</td>
<td>Leloup et al, 2013</td>
</tr>
<tr>
<td>Stationary</td>
<td>20</td>
<td>19</td>
<td>61</td>
<td>Leloup et al, 2013</td>
</tr>
<tr>
<td>Stationary</td>
<td>42</td>
<td>6</td>
<td>52</td>
<td>Qu et al, 2012</td>
</tr>
<tr>
<td>Stationary</td>
<td>24</td>
<td>17</td>
<td>59</td>
<td>Henderson et al, 2008</td>
</tr>
</tbody>
</table>

A summary (Table 2) of overall trends in yield for the C-DBPs indicate a number of key facets:

a) The most abundant data relate to THMs, and trichloromethane (TCM) specifically;

b) The reported TCM yield value for a single species (*Microcystis aeruginosa*) varies by more than a factor of two across the five studies;

c) Most studies have been based on one or two species, rather than a wider range;

d) The chlorination conditions adopted vary between the studies with respect to the to Cl₂:C ratio and exposure time;

e) The limited data available suggests that the phase of the growth cycle may also influence both the amount and the yield of the DBP generated.

Interpretation of the available literature data across different studies is challenged by the different experimental conditions adopted, the differing fractions of the algal matter studied, and the limited scope of the studies in terms of the number of species investigated (predominantly one or two). It is of interest to establish whether any trends or patterns if DBP formation, and yield specifically, exist for AOM across different algal species. AOM is of practical interest since the algal solids are retained by the filtration process, the dissolved AOM component being the fraction subjected to final chlorination. Both C- and N-DBP formation is considered from AOM of six algal species at the onset of the stationary phase. Characterisation
encompasses hydrophilicity, charge, protein and carbohydrate content, with a view to linking character to DBP formation potential with reference to THMs, HAAs, HANs and one HNM (trichloronitromethane, TCNM).

Table 2: Summary of selected published chlorinated DBP yield data

<table>
<thead>
<tr>
<th>Algal species</th>
<th>TCM, µg mg⁻¹ C</th>
<th>DCAA</th>
<th>TCAA, µg mg⁻¹ C</th>
<th>Cl₂:C</th>
<th>t, h</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anabaena flos-aquae</em>₁ᵃ</td>
<td>35</td>
<td>26</td>
<td>22</td>
<td>-</td>
<td>168</td>
<td>Huang et al., 2009</td>
</tr>
<tr>
<td><em>Anabaena flos-aquae</em>₁ᵃ</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>1.4</td>
<td>24</td>
<td>Wachter &amp; Andelman, 1984</td>
</tr>
<tr>
<td><em>Cyclotella meneghiniana</em> b</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>72, 168</td>
<td>Laio et al., 2015</td>
</tr>
<tr>
<td><em>Chaetoceros muelleri</em> a</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>168</td>
<td>Nguyen et al. 2005</td>
</tr>
<tr>
<td><em>Chlamydomonas sp.</em> b</td>
<td>25</td>
<td>213</td>
<td>67</td>
<td>20</td>
<td>120</td>
<td>Lui et al., 2012</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> b</td>
<td>61</td>
<td>-</td>
<td>-</td>
<td>0.4</td>
<td>168</td>
<td>Huang et al., 2009</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> a</td>
<td>35</td>
<td>42</td>
<td>24</td>
<td>-</td>
<td>168</td>
<td>Huang et al., 2009</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> a</td>
<td>16</td>
<td>11</td>
<td>-</td>
<td>5</td>
<td>72</td>
<td>Fang et al., 2010</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> a</td>
<td>27</td>
<td>11</td>
<td>11</td>
<td>3</td>
<td>72</td>
<td>Qi et al., 2016</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> b</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td>7.1</td>
<td>72, 168</td>
<td>Laio et al., 2015</td>
</tr>
<tr>
<td><em>Nitzschia sp.</em> b</td>
<td>48</td>
<td>25</td>
<td>19</td>
<td>10</td>
<td>96</td>
<td>Hong et al., 2008</td>
</tr>
<tr>
<td><em>Oscillatoria prolifera</em> a</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>168</td>
<td>Nguyen et al. 2005</td>
</tr>
<tr>
<td><em>Scevedesmus quadricauda</em> b</td>
<td>48</td>
<td>35</td>
<td>23</td>
<td>5</td>
<td>168</td>
<td>Nguyen et al. 2005</td>
</tr>
<tr>
<td><em>Scevedesmus quadricauda</em> b</td>
<td>64</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>168</td>
<td>Nguyen et al. 2005</td>
</tr>
</tbody>
</table>

Cl₂:C chlorine:carbon mass ratio; t chlorination time; ¹ Exponential growth phase; ² Stationary growth phase; ³ HPO fraction; ⁴ >0.5 mg/L residual; ⁵ 20 mg/L; ⁻ AOM, ᵇ – algal cells

2 Materials and methods

2.1 Algal cultivation

Freshwater algae *Scenedesmus subspicatus* (276/20), *Aphanizomenon flos-aquae* (1401/3), *Anabaena flos-Aquae* (1403/13B) and *Microcystis aeruginosa* (1450/3) *Asterionella Formosa* (1005/9) (CCAP, Scotland) and *Melosira sp.* (JA386) (Sciento, UK) were cultured according to recommended conditions (Table 3). Lighting was supplied by a Sun-glo and an Aqua-glo 30W lamp. Neutral density filters were used with the lights for all species except *Scenedesmus subspicatus*. Each species grew at a different rate and reached the maximum phase of growth with different cell concentrations (Table 3). AOM was extracted from each algal species once exponential growth conditions had been established and at the onset of the stationary phase. Checks were undertaken on a daily basis to ensure contamination had not occurred and to determine cell concentrations: as with previous studies, with cultivation of algae on a similar scale, cultures were only invaded by other organisms in the late stationary/decline phase (Lüsse et al., 1985). Cell numbers were measured in triplicate using a light microscope and haemocytometer.0
Table 3: Algae cell concentrations and time of growth

<table>
<thead>
<tr>
<th>Algal species</th>
<th>Max. cell concentration (cells/ml)</th>
<th>Days taken</th>
<th>Cultivation temperature (°C)</th>
<th>Light/dark cycle (h)</th>
<th>Shaking regime</th>
<th>Growth media</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scenedesmus subspicatus</em></td>
<td>$1.8 \times 10^6$</td>
<td>14</td>
<td>20</td>
<td>16/8</td>
<td>120 rpm</td>
<td>Jaworski</td>
</tr>
<tr>
<td><em>Aphanizomenon flos-aquae</em></td>
<td>$1.8 \times 10^6$</td>
<td>28</td>
<td>20</td>
<td>16/8</td>
<td>120 rpm</td>
<td>Jaworski</td>
</tr>
<tr>
<td><em>Anabaena flos-aquae</em></td>
<td>$8.8 \times 10^5$</td>
<td>30</td>
<td>20</td>
<td>16/8</td>
<td>120 rpm</td>
<td>Blue/green (no N$_2$)</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em></td>
<td>$1.5 \times 10^7$</td>
<td>32</td>
<td>20</td>
<td>16/8</td>
<td>120 rpm</td>
<td>Jaworski</td>
</tr>
<tr>
<td><em>Asterionella Formosa</em></td>
<td>$2.9 \times 10^5$</td>
<td>24</td>
<td>15</td>
<td>14/10</td>
<td>By hand</td>
<td>Diatom</td>
</tr>
<tr>
<td><em>Melosira sp.</em></td>
<td>$1.9 \times 10^4$</td>
<td>8</td>
<td>15</td>
<td>14/10</td>
<td>By hand</td>
<td>Diatom</td>
</tr>
</tbody>
</table>

2.2 AOM extraction and characterisation

AOM was extracted by centrifuging 1 L of algal cell suspension at 4,000 rcf (relative centrifugal force) for 15-30 minutes. The supernatant was filtered with a 0.7 μm glass microfiber filter paper (Fisher Scientific, UK).

Specific ultraviolet absorbance (SUVA) in L m$^{-1}$ mg C$^{-1}$ was determined from the ratio of the 254 nm UV absorbance (m$^1$) to the DOC concentration (mg C L$^{-1}$). UV absorbance was measured using a Jenway 6505 UV/Vis spectrophotometer (Patterson Scientific, UK). The isoelectric point was determined by measuring the zeta potential (mV) over a pH range from 0-10. Zeta potential was measured using a Malvern ZetaSizer 2000 (Malvern, UK). Measurements were carried out in triplicate.

Carbohydrate content was determined using the phenol–sulphuric acid method (Zhang et al., 1999; Dubois et al., 1956). Protein analysis was carried out using the modified Lowry method (Frølund et al., 1995). Glucose and bovine serum albumin were used for calibration with absorbance at 480 nm and 750 nm respectively using the Jenway spectrophotometer. Protein and carbohydrate measurements were triplicated.
The hydrophilicity and hydrophobicity of the AOM samples was determined by fractionation using XAD resins (XAD-7HP and XAD-4) in tandem according to Malcolm and MacCarthy (1992) and reported by Sharp et al. (2006). Charge density (meq g\(^{-1}\)) was measured using a back titration adapted from Kam and Gregory (2001) and described in Sharp et al. (2006).

DOC was measured using a Shimadzu TOC-5000A analyser (Shimadzu, UK) on filtered samples. DOC was calculated by subtraction of the measured inorganic carbon (IC) from the total carbon (TC). The machine was calibrated daily. Up to five replicates were measured and an average of three reported to reduce the coefficient of variance to <2%.

### 2.3 DBP formation and quantification

Chlorination employed a method adapted from standard methods (APHA, 1992). This involved buffering samples at pH 7, adding an excess of free chlorine at 5 mg Cl\(_2\) mg\(^{-1}\) C and storing for seven days at 20°C. Chlorine residuals (measured in the range 0.5-1.2 mg/L) were quenched using 100 mg L\(^{-1}\) ammonium chloride for HAA\(_9\) and HAN\(_4\) and TCNM analysis and 100 mg L\(^{-1}\) sodium sulphite for THM\(_4\) analysis. Additionally THM\(_4\), HAN\(_4\) and TCNM samples were buffered at pH 4.5-5.5.

THM\(_4\) (trichloromethane, dichlorobromomethane, dibromochloromethane, tribromomethane) HAN\(_4\) (bromochloroacetonitrile, dibromoacetonitrile, dichloroacetonitrile, trichloroacetonitrile) and TCNM were extracted using a modified form of USEPA Method 551.1. This method involved salted liquid/liquid extraction with solvent extracts analysed by gas chromatography (GC) with microelectron capture detection (µECD) (Agilent 6890). HAA\(_9\) (monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA), dibromoacetic acid...
(DBAA), bromodichloroacetic acid (DBCAA), dibromochloroacetic acid (DBCAA), and tribromoacetic acid (TBAA)) were analysed using a modified form of USEPA Method 552.3 (Tung et al., 2006). The derivatised HAAs (methyl esters) were measured using GC-µECD. All samples were chlorinated and analysed in duplicate. The limit of quantification for all DBPs was 1 µg L\(^{-1}\), except for MCAA where the quantification limit was 2 µg L\(^{-1}\). DBP yields were calculated by dividing the concentration of DBP (in µg L\(^{-1}\)) by the DOC concentration (in mg L\(^{-1}\)) to give values in µg mg C\(^{-1}\).

3 Results
3.1 AOM characteristics
AOM from all algae characterised was predominantly hydrophilic, as suggested by low SUVA values (0.34-1.7 m\(^{-1}\) L mg C\(^{-1}\)) and verified by the high percentage (from 54% for *Scenedesmus subspicatus* to 81% for the cyanobacteria *Anabaena flos-aquae*) of hydrophilic material (Table 4). This is in accordance with other researchers, for which HPI fractions of 52-73% have been reported (Qu et al., 2012, Henderson et al., 2009). The charge density of all extracted AOM was negligible except for that from the cyanobacteria *Microcystis aeruginosa*, measured at 0.2 meq g\(^{-1}\) and indicating the excreted organics to be predominantly uncharged. The isoelectric point of the AOM samples ranged from 0.9 to 3.2 with the lowest value observed for the AOM from the diatom *Asterionella Formosa*. The protein:carbohydrate mass ratio was similar for the AOM from *Aphanizomenon flos-aquae*, *Anabaena flos-aquae* and *Scenedesmus subspicatus* ranging from 1.1-1.5. In contrast the AOM from *Microcystis aeruginosa* has been reported as having a much lower ratio of 0.4-0.62 (Qu et al, 2012; Henderson et al, 2008).

<table>
<thead>
<tr>
<th>Algal species</th>
<th>SUVA</th>
<th>HPO %</th>
<th>HPI %</th>
<th>Pr/AOM</th>
<th>Ca/AOM</th>
<th>Pr/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aphanizomenon flos-aquae</em></td>
<td>0.79</td>
<td>18</td>
<td>63</td>
<td>0.99</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Anabaena flos-aquae</em></td>
<td>0.34</td>
<td>8</td>
<td>81</td>
<td>0.52</td>
<td>0.34</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Scenedesmus subspicatus</em></td>
<td>1.18</td>
<td>26</td>
<td>54</td>
<td>1.5</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>
The low charge density values indicate diminished quantities of the charged hydrophilic polysaccharides, and the presence of uncharged polysaccharides such as acetylamino sugars, sulphated sugars and carboxylated sugars (Leppard, 1995). These charged hydrophilic polysaccharides have been detected in AOM extracted from the stationary but not the exponential phase (Henderson et al., 2008). The organics excreted from AOM thus comprise low-SUVA organics such as hydrophobic proteins and uncharged hydrophilic polysaccharides (Edzwald, 1993), as well as proteins, peptides, carbohydrates and possibly amino acids (Bond et al., 2009, Pivokonsky et al., 2014).

### 3.2 DBP formation

#### 3.2.1 Trihalomethanes

Under the chlorination conditions adopted, and specifically the absence of bromide, TCM accounted for more than 99% by mass of the THMs formed from the AOM for all six algal species studied (Figure 1). *Aphanizomenon flos-aquae*-AOM followed by *Microcystis aeruginosa*-AOM formed the most TCM of all the species measured at 56.6±3.6 µg mg\(^{-1}\) C and 42.6±3.3 µg mg\(^{-1}\) C respectively. The remaining four AOM samples formed similar levels of THMs between 18.7±2.5 µg mg\(^{-1}\) C and 26.6±4.3 µg mg\(^{-1}\) C. The data complements that from previous studies (Table 1), with similar levels for *Microcystis aeruginosa*-AOM (Huang et al., 2009, Fang et al., 2010) and *Anabaena flos-aquae*-AOM (Huang et al., 2009; Wachter and Andelman, 1984). In contrast, THM formation reported for *Scenedesmus quadricauda*-AOM by Nguyen et al. (2005), referring to AOM extracted during stationary phase, varied depending on the algal growth tank size from 48±12 to 64±14 µg mg\(^{-1}\) C, significantly higher than the 19.9±7.5 µg mg\(^{-1}\) C measured in the current study. Algae grown under the same conditions and from the same tank have been shown to exhibit different behaviour depending on the algal type. The THM yield can vary with growth phase (*Anabaena flos-aquae*-AOM,
Huang et al., 2008) but has also been shown not to vary significantly with growth phase when
normalised with respect to DOC (Scenedesmus quadricauda-AOM, Nguyen et al., 2005;
Microcystis aeruginosa-AOM, Huang et al, 2009).
Comparison with alternative OM sources reveals that AOM exerts a moderate to low reactivity
with chlorine. For instance THM yield concentrations generated from NOM formation
potential tests have been reported to range from 20-281 µg mg⁻¹ C with a median of 63 µg mg⁻¹
¹ C for a range of 35 water sources (Allgeier and Summers 1995, Afcharian et al., 1997, Collins
et al., 1986, Nokes et al., 1999, Singer et al., 1995, Teksoy et al., 2008, Yang et al., 2015, Pifer
and Fairey 2014). This indicates that although AOM may not be the biggest contributor to the
formation of THMs compared to NOM, it could still make a significant contribution to the
THMs formed.
Further to this, microbially derived OM has been shown to exhibit a yield of 23-43 µg THMs
mg⁻¹ C (Sirivedhin and Gray, 2005), with the yield reported to vary little across the three
chemical fractions (Zhou et al, 2014). AOM is known to most resemble hydrophilic NOM and
microbially derived OM and consists of hydrophilic polysaccharides and hydrophobic proteins
(Henderson et al., 2008), so a comparison can therefore be made with the yield of proteins and
carbohydrates. The THM yield has been reported to range from 41 to 51 µg THM mg⁻¹ C for
four proteins (Scullly et al., 1988), and carbohydrates have been observed to form similar levels
of THMs (42 to 65 µg THM mg⁻¹ C) for 10 carbohydrates (Navalon et al., 2008), broadly
consistent with the trends shown in Figure 1.
3.2.2 Haloacetic acids

As with the THM data, brominated species did not feature amongst the HAAs assayed. DCAA and TCAA comprised more than 99% of the total HAAs formed on a mass basis for the AOM of all 6 species of algae (Figure 2), consistent with Nguyen et al. (2005). *Scenedesmus subspicatus*-AOM formed the most HAAs of all the species at a yield of 35.8±2.3 µg mg\(^{-1}\) C followed by *Microcystis aeruginosa*-AOM with yield of 28.7±7.5 µg mg\(^{-1}\) C. AOM from *Aphanizomenon flos-aquae* and *Asterionella formosa* was comparable in HAA yield with values of 24-25 µg mg\(^{-1}\) C ± ~20%. The second-lowest HAA yield was observed for *Anabaena flos-aquae*-AOM at 18.7±1.3 µg mg\(^{-1}\) C with the lowest value of 13.2±2.3 µg mg\(^{-1}\) C recorded for *Melosira sp.*-AOM. As with the THM yield values, those for HAAs measured by Nguyen et al. (2005) from AOM from the stationary phase were higher than those observed in the current study for *Scenedesmus*-AOM (60±7.7 compared to 35.8±3.4 µg mg\(^{-1}\) C) when the AOM was taken at the onset of the stationary phase. This was also the case with values reported from
the stationary phase by Huang et al. (2009) compared to those observed in the current study (66 compared to 29 µg mg\textsuperscript{-1} C for *Microcystis aeruginosa*-AOM, and 48 compared to 19 µg mg\textsuperscript{-1} C for *Anabaena flos-aquae*-AOM). The higher yield from *Microcystis aeruginosa*-AOM compared to *Anabaena flos-aquae*-AOM (Figure 2) corroborates the findings of Huang et al. (2009), attributable to the difference in HPO content (Table 4).

![Figure 2: HAA concentrations produced by the AOM from each algal species](image)

The TCAA:DCAA ratios observed in the current were comparable to those reported in the literature for *Scenedesmus*-AOM: 0.60 compared to 0.33-0.69 reported by Nguyen et al. (2005) over a number of days of stationary growth. However the same ratios reported by Huang et al. (2009) for AOM from *Microcystis aeruginosa* and *Anabaena flos-aquae* (0.57 and 0.85 respectively) extracted during the stationary phase were significantly lower than those from the current study (2.1 and 7.3 respectively) for samples taken at the onset of the stationary phase. The difference may be attributable to the varying amino acid content, which can have wide
ranging HAA yield values - insignificant to 106 µg mg\(^{-1}\) C according to Hong et al., 2009 - and may consist largely of aromatic/cyclic amino acids (Bond et al., 2009).

HAA formation was positively correlated HPO (\(R^2 = 0.94\)) which was attributed mainly to DCAA formation. Conversely the hydrophilic content was negatively correlated to HAA formation (\(R^2 = 0.87\)), again closely linked to DCAA formation.

### 3.2.3 Haloacetonitriles

Dichloroacetonitrile (DCAN) comprised >99% of the total HANs formed on a mass basis for the AOM for all 6 algal species (Figure 3). Trichloroacetonitrile (TCAN) was not detected in any samples likely due to base-catalysed hydrolysis at pH>5.5 (Croué and Reckhow, 1989). *Microcystis aeruginosa*-AOM, *Scenedesmus subspicatus*-AOM and *Melosira sp.*-AOM generated the highest HAN yields of all the species measured at 1.32±0.01, 1.10±0.07 and 0.87±0.05 µg mg\(^{-1}\) C respectively. *Aphanizomenon flos-aquae*-AOM produced the lowest yields (0.12±0.003 µg mg\(^{-1}\) C), with *Asterionella formosa*-AOM and *Anabaena flos-aquae*-AOM exhibiting similar values of 0.53±0.07 and 0.39±0.10 µg mg\(^{-1}\) C respectively. The formation potential for HANs from AOM has been studied by Fang et al. (2010) and for fractionated AOM (Zhou et al., 2014). These authors reported slightly higher values of ~1.5 µg mg\(^{-1}\) C DCAN from chlorination of *Microcystis aeruginosa*-AOM compared to the current study, perhaps because the AOM was extracted during the stationary phase. For fractionated samples, values of total HANs from chlorination of *Microcystis aeruginosa*-AOM over 3 days ranged from 1.5–2.6 µg mg\(^{-1}\) C, with the HPO fraction having the greatest formation potential (Zhou et al., 2014). This equates to a value of 1.8 µg mg\(^{-1}\) C, based on the relative amount of each fraction, which is slightly higher than the values found in the current study for *Microcystis aeruginosa*-AOM but does not take into account the synergistic effects encountered when chlorinating non-fractionated samples (Kent et al., 2011). HAN yields from algal cells have
been reported under similar chlorination conditions, albeit with a 3-day exposure, of 0.76 µg mg\(^{-1}\) C DCAN and 0.05 µg mg\(^{-1}\) C TCAN from algal cell suspensions of *Microcystis aeruginosa* (Fang et al., 2010). Under the same chlorination conditions as reported here, at double the chlorine dose, Oliver (1983) reported DCAN formation of 2.3 µg mg\(^{-1}\) C and 0.5 µg mg\(^{-1}\) C for cyanobacterial (*Anabaena Texas 1447*) and green (*Scenedesmus basiliensis*) algal suspensions respectively.

![Figure 3: DCAN concentrations produced by the AOM from each algal species](image)

In terms of THM and HAA formation, algal cells have been observed to produce similar or greater amounts than the corresponding AOM (Huang et al., 2009). Differences in algal species, organic fractions (dissolved matter vs. whole cells), and chlorination conditions make comparison with published challenging. However, the values for AOM reported in the current study are of the same magnitude as those reported in the literature for algal cell suspensions and less than the yield of DCAN from isolated fulvic acid (4.3 µg mg\(^{-1}\) C) (Oliver, 1983).
Under identical chlorination conditions, Lee et al. (2007) reported the chlorination of isolated NOM fractions to produce DCAN levels ranging from 1.65 to 2.31 µg mg\(^{-1}\) C from TPI neutral and colloidal fractions largely consisting of amino sugars, polysaccharides and proteins. Contrary to Oliver (1983), HPO fractions (including fulvic acid) produced DCAN levels of 0.33-0.77 µg mg\(^{-1}\) C (Lee et al., 2007) which could be related to the differing chlorine doses.

3.2.4 Halonitromethane

TCNM was the only HNM measured in the current study (Figure 4). *Melosira sp.*-AOM formed the most TCNM (0.36±0.02 µg mg\(^{-1}\) C) of all the species measured at followed by *Asterionella formosa*-AOM (0.24±0.03 µg mg\(^{-1}\) C). Similar values were observed for *Anabaena flos-aquae*-AOM, *Microcystis aeruginosa*-AOM and *Aphanizomenon flos-aquae*-AOM forming 0.16±0.01 µg mg\(^{-1}\) C and 0.13±0.01 µg mg\(^{-1}\) C and 0.11±0.01 µg mg\(^{-1}\) C TCNM respectively. TCNM formation by *Scenedesmus subspicatus*-AOM was below the limit of detection.

![Figure 4](image-url)  
**Figure 4:** TCNM concentrations produced by the AOM from each algal species compared to literature values
To the authors’ knowledge the formation potential for HNMs from AOM or algal cells has only been studied for *Microcystis aeruginosa*-AOM (Fang et al., 2010) with slightly higher values than observed here perhaps expected due to the difference in growth phase as described for the other DBPs measured. Values of TCNM reported from chlorination of isolated NOM fractions average at 0.33 µg mg⁻¹ C (Lee et al., 2007) similar to the values reported here.

4 Discussion

AOM is mainly hydrophilic in character and on chlorination has the potential to form significant amounts of C- and N-DBPs. An unsuccessful attempt was made to link the characteristics to the DBPs formed as this has been shown to be applicable for NOM and DBPs (e.g. with NOM, THM formation can correlate positively with SUVA for a range of water samples for high SUVA (>3) waters (Ates et al., 2007, Reckhow et al., 1990). No relationship was observed between SUVA and the DBPs measured in the current study and, apart from the close correlation of HAA with the HPO fraction (Section 3.2.2), there was no correlation evident between DBPFP and any chemical fraction.

No pattern in DBP formation with algal taxonomic group was evident. For instance, AOM could form significant amounts of C-DBPs (illustrated by the specific cyanobacterial species AOM and green algal AOM) or less significant amounts of C-DBPs (illustrated by the AOM from diatomaceous species). Cyanobacterial AOM may be expected to produce significant amounts of nitrogenous DBPs compared to green and diatomaceous AOM since cyanobacterial algae are nitrogen fixers and liberate up to 45% of their fixed nitrogen as organic-N (Huang et al., 2009, Westerhoff and Mash, 2002). Indeed, when looking at formation of HANs, one particular cyanobacteria is more reactive (*Microcystis aeruginosa*-AOM) but significant
amounts of HAN are also formed by green and diatomaceous AOM. The AOM from the
diatoms (*Melosira sp.* and *Asterionella formosa*) forms the most TCNM followed by the
cyanobacterial AOM, with no formation of TCNM by the green AOM (*Scenedesmus
subspicatus*). Therefore when considering the risk of DBPs formed by a particular algal species,
it is important that the AOM produced from diatomaceous algae is considered as it can be a
significant precursor to HAN and HNM formation.

The chlorination of AOM involves the reaction between chlorine and molecules including
uncharged hydrophilic polysaccharides, proteins, peptides and carbohydrates. The amino acids
present in freshwater algae (as free amino acids and proteins or peptides) comprise at least 17
of the 20 standard amino acids (Fowden, 1951, Lewis and Gonzalves, 1962) with different
species containing different amino acids. For example, some cyanobacterial algal species
contain no cysteine whereas some green algal species contain cysteine but no lysine. All the
amino acids present in algae can potentially be present in AOM. It is known that acid
polysaccharides such as uronic acids can be excreted by algae in response to low nutrient stress
(Costerton, 1984). The polysaccharides present in algae and extracellularly comprise the
carbohydrates rhamnose, galactose, arabinose, fucose, mannose, glucuronic acid, uronic acid,
glucose (Rezanka and Sigler, 2007).

Amino acids, carbohydrates and carboxylic acids have been studied with respect to their DBP
formation (Chu et al., 2012, Shan et al., 2011, Bond et al., 2009, Navalon et al., 2008, Trehy et
al., 1986). The study of carbohydrates (Navalon et al., 2008) showed that they were reactive
with respect to THM (40-65 µg mg⁻¹ C). In studies of amino acids, the key finding was that the
compounds can have similar physicochemical properties but divergent DBP formation. For
example, glutamic and aspartic acid have very similar log *K*<sub>ow</sub>, pKa, and molecular weight.
However, on chlorination aspartic acid forms DCAA, trichloroacetaldehyde, and DCAN at 0.26, 0.02, and 0.06 mol THM/mol compound (mol/mol) respectively, whereas none of these species are formed from glutamic acid chlorination (Bond et al., 2009). On the other hand, little difference was observed between formation of HNM (Shan et al., 2011) and DCAN (Wang et al., 2013) from glutamic and aspartic acids, emphasising the different pathways of formation for each group of DBPs. Mechanisms of formation have been proposed for these pathways (Table 5). A study on carboxylic acids (Bond et al., 2009) showed that β-dicarbonyl 3-oxopentanedioic is reactive with respect to THM and trichloropropane formation but not HAA formation. This corroborated a previous report stating that the reactivity of carbohydrates and carboxylic acids towards chlorine to be low (WHO, 2000) with reference to the chlorine demand of the carbohydrates, though this report did not consider that significant amounts of some DBPs could still be formed.

Table 5: Proposed pathways for DBP formation from amino acid precursors

<table>
<thead>
<tr>
<th>DBP</th>
<th>Precursors</th>
<th>Intermediate</th>
<th>Substitution location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNMs</td>
<td>Chemical structure of precursors not considered to be important</td>
<td></td>
<td></td>
<td>Wang et al., 2013, Shan et al., 2011</td>
</tr>
<tr>
<td>HANs</td>
<td>Aspartic acid, asparagine</td>
<td>dichlorocyanacetic acid</td>
<td>nr</td>
<td>Wang et al., 2013</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td>benzyl cyanide</td>
<td>α-carbon</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Histidine</td>
<td>2-(1-chloro-1H-imidazol-4-yl)-acetonitrile</td>
<td>α-carbon</td>
<td>Li and Blatchley, 2007</td>
</tr>
<tr>
<td>THMs</td>
<td>Tyrosine</td>
<td>4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol</td>
<td>nr</td>
<td>Chu et al., 2012</td>
</tr>
<tr>
<td>HAAs</td>
<td>Aspartic acid and glutamic acid</td>
<td>β-keto acid such as 3-oxopentanedioic acid or cyanoacetic acid</td>
<td>Variable</td>
<td>Bond et al., 2009</td>
</tr>
</tbody>
</table>

nr – not reported

While AOM is present at lower concentrations than other DBP precursors such as NOM, its nature means that it is recalcitrant to treatment by methods such as coagulation. Whilst optimised coagulation has been shown to remove the algae *C. Vulgaris, M. aeruginosa* and *A.*
Formosa by 71, 55 and 46% respectively (Henderson et al., 2010), the removal of dissolved AOM is more challenging due to its uncharged hydrophilic nature; enhanced techniques such as pre-ozoneation demonstrating only partial success (Widrig et al., 1996). Given the escalation of eutrophication of water sources in recent years due to anthropogenic effects, increasing the levels of phosphorus and nitrogen entering water sources (Ward and Wetzel, 1980, Burrini et al., 2000) AOM is likely to be a significant contributor to DBP formation in treated drinking waters.

Another important consideration is the toxicity of the DBPs formed, particularly the nitrogenous DBPs. A recent study (Zeng et al., 2016) on potable water reuse investigated a range of DBPs throughout the treatment train and looked at the contribution of each DBP to the toxicity of the water. The toxicity was determined as a function of concentration and toxic potencies of each DBP. The toxicity in this case for unregulated halogenated DBPs was based on in vitro chromic cell cytotoxicity which has some limitations and the authors stressed that they were determining relative rather than absolute risk. Nonetheless they found that HANs, haloacetamides and to a lesser degree haloacetaldehydes dominated the additive toxicity in membrane filtrate. Thus it is important, when considering whether to use an algal impacted source, that the concentration of nitrogenous DBPs (particularly HANs and haloacetamides) in the treated water may be elevated compared to a source that is not algal impacted.

5 Conclusions

A study of the characteristics of formation of chlorinated disinfection by products from algal organic matter (AOM) has revealed the following:
• AOM is mainly hydrophilic in character, with between 52 and 81% being made up of HPI fraction, and on chlorination has the potential to form up to 92.4 µg carbonaceous and 1.7 µg nitrogenous DBPs per mg organic carbon;

• No pattern in DBP formation with algal taxonomic group was evident;

• Few consistent trends between DBP formation propensity and either the specific ultraviolet absorbance (SUVA) or the AOM chemical characteristics were evident, such that characterisation of the AOM may be of limited use in determining DBP formation;

• Although little studied, the AOM from diatomaceous algae forms significant amounts of nitrogenous DBPs (up to 1.7 µg mg\(^{-1}\) C).

The hydrophilic nature of AOM, which is autochthonous in nature, makes it more difficult to remove effectively using conventional water treatment processes than allochthonous natural organic matter (NOM), which is also more hydrophobic in nature. This offers an explanation for the generally observed trend of seasonally high chlorinated DBP levels associated with higher temperatures and thus commensurately greater microbial production rates with accompanying AOM generation.

References


Chu, W., Gao, N., Krasner, S.W., Templeton, M.R., Yin, D., 2012. Formation of halogenated C-, N-DBPs from chlor(am)ination and UV irradiation of tyrosine in drinking water. Environmental Pollution 161, 8–14.


